

Nucleus pulposus contain progenitor-like cells able to differentiate into osteogenic and adipogenic lineages *in vitro*

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INTRODUCTION: The intervertebral disc (IVD) has a limited regenerative potential and low back pain represents a leading cause of disability [1]. IVD repair strategies require an appropriate cell source that is able to regenerate the damaged tissue such as progenitor stem cells. Recently, progenitor cells that are positive for the angiopoietin receptor (Tie2) in the nucleus pulposus were identified [2]. Here we isolated primary cells from bovine IVD and sorted bovine nucleus pulposus progenitor cells (NPPC) for the marker Tie2. Furthermore, we tested whether Tie2 expressing cells can differentiate into osteogenic and adipogenic lineages *in vitro*.

METHODS: NP cells were obtained from one year old bovine tails by sequential digestion with pronase for 1 hour and collagenase overnight. Sorted Tie2- and Tie2+ cells were cultured in osteogenic and adipogenic medium for 3 weeks. The formed cell layers from both subpopulations were stained for calcium deposition and fat droplets. Colony forming units were prepared for both cell suspensions in methylcellulose-based medium and formed colonies (> 10 cells) were analyzed macroscopically after 8 days.

RESULTS: After 3 weeks of culture, sorted Tie2+ cells were able to differentiate into osteocytes and adipocytes as characterized by calcium deposition and fat droplet formation. By contrast, Tie2- cells generated a weak staining for calcium and no fat droplets were obtained (Figure 1). Sorted Tie2- and Tie2+ subpopulations of cells both formed colonies, however with different morphologies. The colonies formed from Tie2+ cells were spheroid in shape whereas those from Tie2- cells were spread and fibroblastic.

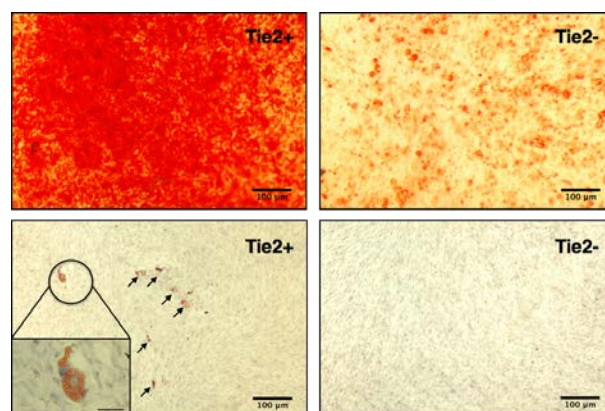


Fig. 1: Differentiation assays of Tie2+ and Tie2- bovine nucleus pulposus cells: Osteogenesis (top row) and adipogenesis (lower row).

DISCUSSION & CONCLUSIONS: Our data showed that Tie2+ cells of the nucleus pulposus cells are progenitor-like cells that are able to differentiate into osteogenic and adipogenic lineages. Sorting of NPPC for Tie2 may represent a promising strategy with the potential to be used in the clinics for treatment of intervertebral disc damage.

REFERENCES: ¹ Freemont AJ. *Rheumatology (Oxford)* 2009; 48:5-10. ² Sakai D, Nakamura Y, Nakai T, et al. *Nat Commun* 2012; 3:1264.

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